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Food Aversion Learning by the Hermit Crab *Pagurus granosimanus*

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Abstract. The common intertidal hermit crab *Pagurus granosimanus* learns in one or two trials to reject an attractive, novel food (beef) when illness is induced by lithium chloride injected one hour after the animal accepts and eats the beef. Crabs fed a familiar food (fish) before lithium chloride injection do not learn to avoid the fish. Nor do they learn to reject beef when injected with a sodium chloride solution, or when punctured with a hypodermic needle one hour after their first and second beef meals. Because many crustaceans are scavengers and generalist feeders, they must commonly encounter a wide variety of toxic foods. Quickly acquired and long-lasting aversion to a new food eaten a few hours before the onset of a serious physiological upset could cause these animals to avoid such hazardous foods in the future. Food aversion learning has never before been reported in a crustacean.

Introduction

From Baja California to Alaska, the common intertidal hermit crab *Pagurus granosimanus* lives on rocky substrates between −1.0 and +0.8 meters, relative to mean lower low water (Nyblade, 1974; Abrams, 1987). Like most hermit crabs, *P. granosimanus* is an omnivorous detritivore that feeds actively on a wide range of plant and animal foods (Orton, 1927; Roberts, 1968; Hazlett, 1981). For an opportunistic feeder living on wave-swept shores, the particular food available, its nutritional value, and the risk of toxicity can vary seasonally, from place to place, and even from tide to tide. This should favor the evolution of sensory capacities and learning mechanisms that allow the animal to be both selective and flexible in its choice of foods.

Food aversion learning is a kind of associative learning that is particularly appropriate for opportunistic feeders (Wilcoxon *et al.*, 1971; Garcia *et al.*, 1974; Garcia and Hankins, 1977; Gustavson, 1977; Zahorik and Houpt, 1981). It is distinguished from classical or operant conditioning on the basis of several distinctive characteristics (reviewed and discussed in Barker *et al.*, 1977). (1) One or a very few conditioning trials are commonly effective. (2) Learning can occur in spite of long delays between ingestion and the resulting illness. (3) The resulting aversion has a long extinction time. (4) Only particular aspects of the food are associated with the illness. (5) Novel foods are much more readily associated with the sickness than are familiar foods.

As generalist feeders and scavengers that distinguish foods primarily by chemoreception (Hazlett, 1968, 1971; Zimmer-Faust, 1987), hermit crabs may benefit from a learning mechanism similar to the taste aversion learning of rats. We demonstrate here that hermit crabs (*Pagurus gransimanus*) quickly learn to reject a novel and attractive food when severe illness is induced by lithium chloride injected about an hour after they first eat that food.

Materials and Methods

Large animals (wet weight 0.48–1.65 grams without the shells) were collected from rockpools at Cattle Point on San Juan Island, Washington, and held in aquaria supplied with running seawater at the Friday Harbor Laboratories. After removing the apex of each shell with a belt sander, we divided the crabs haphazardly into 6 groups of 15 animals each. Each group was held in a plastic mesh (Vexar) cage divided into separate 10 cm square compartments for each animal. The cages were raised 4 cm off the bottom of the aquaria so the animals could not browse on accumulated detritus.

The foods used were fresh ground beef and fresh fish

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(sole) that were frozen raw. Only the amount needed was thawed each day to maintain equal freshness throughout the experiment. The crabs were hand-fed twice a day (morning and evening): we offered them tiny pieces of freshly thawed food on the end of a dissecting probe. Uneaten food that fell through the plastic mesh was removed from the aquaria half an hour later. The crabs were fed fish for at least two days before treatments began. On treatment days we fed them, moved them into separate finger bowls an hour later, and removed them from their shells by gently prodding the abdomens with a thin piece of plastic coated wire inserted through the hole at each shell apex.

Injections were done with a microliter syringe with a fixed needle that was wiped with alcohol between injections. Ten-microliter doses were injected into the thorax dorsally at the joint between the thorax and abdomen. Solutions used were 1.1 *M* lithium chloride (LiCl) and 1.1 *M* sodium chloride (NaCl) in glass distilled water.

On the first day of the experiment (day 0), four of the six groups were fed *beef*: of these, one group was injected one hour later with lithium chloride (LiCl), a second with sodium chloride (NaCl), the third merely pierced with the hypodermic needle but not injected, and the fourth only removed from the shell. The two remaining groups were fed *fish;* and one hour later one group was injected with lithium chloride, and the other with sodium chloride.

Only animals that accepted the test food when it was next offered (24 h after the first treatment) received a second treatment on the following day (day 1).

The crabs' responses to food were tested twice daily for the next 11 days (days 2–12) without further treatment. They were offered bits of fish in the morning and beef at night on the tip of a probe, and each animal's response was scored as either acceptance or rejection (described in the results below).

To reduce the amount of handling during treatment, the animals were not weighed initially. Instead, on day 10 of the experiment, surviving animals were removed from their shells and weighed individually to the nearest hundredth of a gram.

Results

Food acceptance and rejection responses

When accepting food from a probe, hermit crabs usually touch the probe with the second antennae or the dactyls of the walking legs, grasp the food using the chelipeds and sometimes also with the walking legs, then pass it toward the mouth, usually using the minor, left cheliped. Both chelipeds may be used to tear off bits that can be ingested, or the whole mass may be manipulated and held against the inner mouthparts by the third maxillipeds.

When rejecting the food, the crabs generally flick the second antennae back and away after contacting the probe. Sometimes they push the food away vigorously with the chelipeds and back away; and sometimes they hesitantly grasp it with the minor cheliped, pass it to the mouthparts, manipulate it for a few seconds, and then eject it forward and upward using a jet of water.

Dosage and effects of lithium chloride

The mean wet weight overall for the animals was one gram (sd = 0.3, n = 89). To avoid excessive handling, the animals were each given the same size injection; and thus the per weight dose of LiCl varied from 250 to 970 mg/Kg wet weight (per treatment).

This dose of LiCl caused limb trembling, uncontrolled movement, and periods of immobility when the animals usually lay on their backs. All of these animals found and reaccepted their shells within two to three hours. The crabs that were injected with NaCl tended periodically to curl tightly into a ball, sometimes remaining immobile for several minutes; but they reaccepted their shells within half an hour. Those that were stabbed with the needle but not injected returned to their shells within 15 minutes with only occasional periods of immobility; and the crabs that were only removed from their shells usually reaccepted them immediately.

Induced aversion to a novel food

All of the animals injected with LiCl after their first encounters with beef developed an aversion to beef (Figs. 1A, 3A). Two-thirds refused beef after only one LiCl injection. The five that were injected again, after their second beef meal, all refused beef when it was offered for the third time. Without additional injections, the number refusing beef continued to be significantly higher than for the controls through day thirteen (G-test with the Williams correction, $P < .05$). On day 14 the number that refused beef was not significantly higher than in the control groups (G-test, $P > .50$).

As individuals, these animals were also more consistent in refusing beef than were the animals in other treatment groups (Fig. 3). Extinction of the response generally required more than a week—on average, the beef-LiCl treated animals refused beef for 6.9 ± 3.0 consecutive days within the first 11-day period following treatment (days 2 through 12), (Fig. 3A). This was significantly longer than for any other treatment group (t-test, $P \ll .001$). This group rejected beef more consistently than it rejected fish (t-test, $P \ll .001$).

Although about twice as many animals in the beef-NaCl and beef-puncture control groups received a sec-

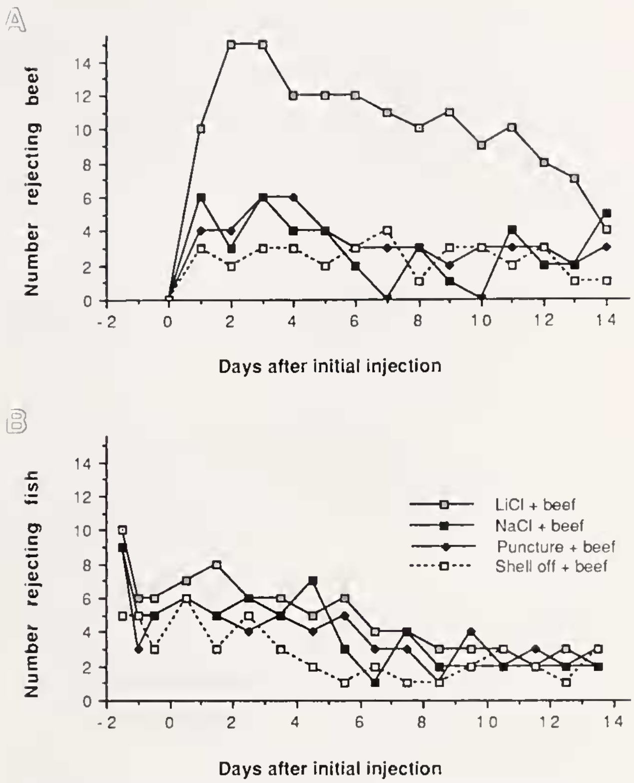

Figure 1. Number of individual hermit crabs (*Pagurus granosimanus*) that rejected daily feedings of (A) beef and (B) fish. The legend applies to both graphs. Four groups of 15 animals each were treated on day zero, one hour after eating a novel food (beef); those that accepted beef the next day received a second treatment. One treatment group was only removed from the shell; a second was also punctured with a hypodermic needle. Two other groups received injections, one with lithium chloride, and the other with sodium chloride.

ond treatment, neither group developed an aversion to beef (Fig. 1A). Significantly more of the animals in the fish-NaCl treatment group rejected beef on first encountering this new food, 12 h after their treatments on day 0 and day 1 (comparison with the beef-shell removal control group; G-test, $P < .01$ and $P < .025$ for day 0 and day 1, respectively; Fig. 2B); however, none of these animals showed a long-term aversion to beef (Fig. 3C).

Consistent acceptance of a familiar food

All of the groups continued to accept fish throughout the test period (Fig. 1B). Of the two groups that were injected after eating this familiar food, neither learned to reject fish (Fig. 2A).

Mortality

Six animals died during the experiment: one from the beef-LiCl group on day 4; three from the fish-NaCl group on days 2, 9, and 10; one on day 11 from the group that was simply removed from the shell; and one from the fish-LiCl group on day 10.

Discussion

When injected with LiCl one hour after their first beef meals, hermit crabs (*Pagurus granosimanus*) learned in one or two trials to avoid this novel food while continuing to eat a familiar food (fish). This aversion to beef commonly lasted for more than a week under laboratory conditions. Hermit crabs rely strongly on chemoreception in locating food (Hazlett, 1968; Zimmer-Faust, 1987). Crustaceans can learn using chemoreception as a cue. Fine-Levy *et al.* (1988) found that the spiny lobster can learn to associate a particular smell with the presence of a predator. It is likely, then, that food is identified and avoided on the basis of chemoreception in response either to water-borne chemicals (smell) or to direct contact (taste). Further work is required to determine what specific food cues are used in this learned avoidance of a specific food.

In laboratory experiments with vertebrates (reviews in

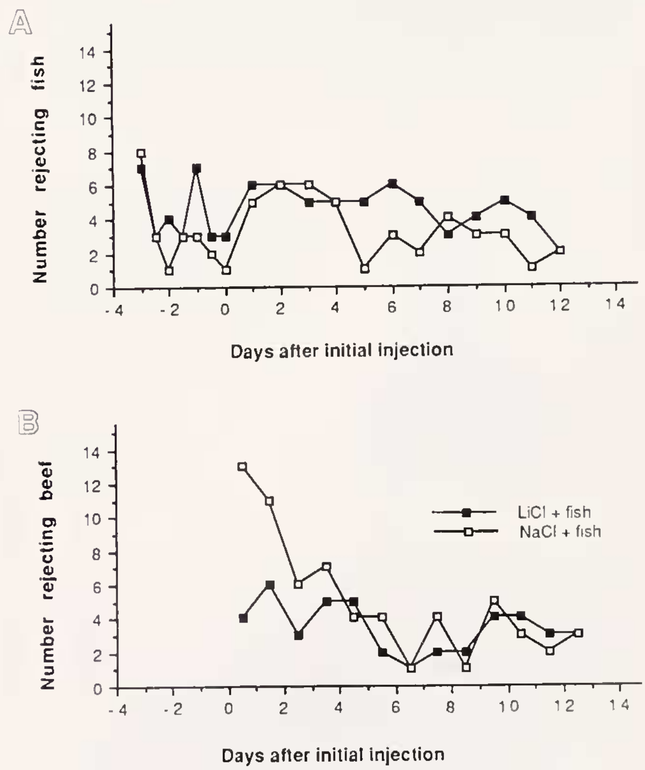

Figure 2. Number of individual hermit crabs (*Pagurus granosimanus*) that rejected (A) fish and (B) beef on each day following treatment. Animals were treated on day zero, one hour after eating a familiar food (fish); those that accepted fish the next day were given a second treatment. The two treatment groups of 15 animals each were injected either with lithium chloride or with sodium chloride.

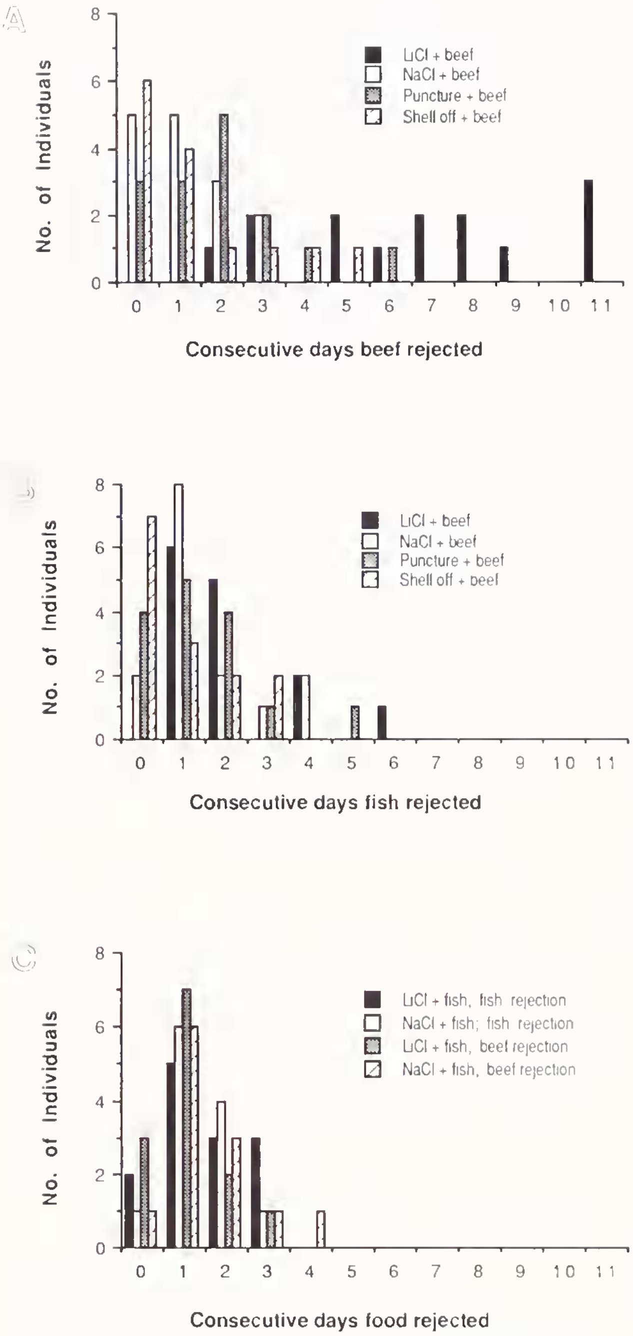

Figure 3. Consistency of food rejection by individual hermit crabs (*Pagurus granosimanus*) in six treatment groups (identified in the legends). Columns show the maximum number of consecutive days that each animal rejected a particular food during the eleven days following treatment: (A) rejection of a novel food (beef) by animals treated one hour after their first beef meal (or after the first and second beef meals, for those that accepted beef on the day after their first treatment), (B) fish rejection by the same animals, and (C) fish and beef rejection by different animals that were treated after eating a familiar food (fish).

Barker *et al.*, 1977), the effects of LiCl injection are generally assumed to mimic the symptoms of illness caused by ingesting a toxic substance. The doses used in our study caused fairly severe and long-lasting general symptoms. Moreover, the food aversion was clearly caused by the effects of the LiCl, and not by osmotic shock or the tonic effects of the injection, because the group injected with the same molar concentrations of NaCl after first eating beef did not develop an aversion to beef. Because animals that developed an aversion to beef continued to accept fish, the aversion is specific, and not merely a generalized, post-trauma avoidance of food. Nor can it be explained as non-specific neophobia (general avoidance of unfamiliar food after an illness), because most of the specimens injected with lithium after a fish meal accepted beef within a few days.

One of the two control groups injected with NaCl solution showed significantly increased rejection of beef for two days following treatment. This response is puzzling because it was inconsistent (*i.e.*, it did not occur in both NaCl injected groups), and because none of the obvious explanations seem to fit. The animals in this group were not significantly smaller than in the other sodium-injected group, which rules out the possibility of unusually high osmotic or tonic stress. If the response were due to non-specific neophobia caused by the treatment, the group injected with lithium after eating fish should also have rejected the beef; but they did not. Whatever the cause of this transient reaction to a new food, it clearly is not long-lasting food aversion of the kind shown by the beef-LiCl treatment group.

Food aversion learning is known to occur commonly among vertebrates (Garcia *et al.*, 1974; Gustavson 1977), and has also been described for a mollusc (Gelperin, 1975) and two insects (Dethier, 1980; Bernays and Lee, 1988). Characteristic of this type of associative learning (Garcia and Hankin, 1977) is rapid aversion to a new food (one or two trials in this case) despite a considerable time lag between ingestion and the onset of illness (in this case, an hour). Also typically, a novel food (in this case, beef) is more readily associated with subsequent internal disorders than is a familiar food (fish). Relatively long extinction times (one or two weeks, here) are also typical.

Most hermit crabs are omnivorous detritivores feeding on fine particles from the sediment as well as on larger morsels of animal matter (Orton, 1927; Roberts, 1968). Thus they are undoubtedly exposed to a wide variety of foods, and presumably also to a wide spectrum of toxins, including rotting debris that can be infested with toxic microorganisms, and macroorganisms that can manufacture or sequester toxins. While food aversion learning has never before been described among crustaceans, many (including the hermit crabs) can learn by classical conditioning (review by Corning *et al.*, 1973). The ability to associate delayed illness with a particular food could be quite advantageous, and might be rather common among the Crustacea.

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